

**THE SAME CLAIM
BUT HAVE EXAMINERS' HANDWRITING ON IT**

CLAIMS

1. A method for obtaining transgenic plants having an increased capacity to synthesize, to accumulate and to exude organic acids, by integration into their genome of a recombinant heterologous DNA molecule encoding enzymes that synthesize organic acids, involving the following steps:
 - (a) preparation of a recombinant heterologous DNA molecule encoding one or more genes for enzymes that synthesize organic acids, linked to a promoter sequence functional in plants, and to a transcription termination/polyadenylation sequence functional in plants;
 - (b) the transformation of plant cells with the recombinant DNA molecule, and
 - (c) the regeneration of transgenic plants starting from transformed cells, or of seeds from plants obtained from these transformed cells, for one or several generations, wherein the genetic information of these transformed cells, includes the recombinant DNA molecule coding for enzymes that synthesize organic acids.
 2. The method according to claim 1, in which the recombinant DNA molecule comprises one or more microbial genes coding for enzymes that synthesize organic acids.
 3. The method according to claim 1, wherein the recombinant DNA molecule comprises a gene of plant origin coding for an enzyme that synthesizes organic acids.
 4. The method according to claim 1, wherein the recombinant DNA molecule comprises a gene of animal origin coding for an enzyme that synthesizes organic acids.
 5. The method according to claim 2, wherein the recombinant DNA molecule comprises one or more bacterial genes that code for an enzyme that synthesizes organic acids.
 6. The method according to claim 1, wherein the recombinant molecule comprises a gene that codes for the enzyme Citrate Synthase.
 7. The method according to claim 1, wherein the recombinant molecule comprises a gene that codes for the enzyme Malate dehydrogenase.
 8. The method according to claim 1, wherein the enzyme that synthesizes organic acids is located in the cytoplasm.

9. The method according to claim 1, wherein the enzyme that synthesizes organic

¹⁰³
+Chou acids is located in chloroplasts.

10. The method according to claim 1, wherein the enzyme that synthesizes organic

¹⁰³
+Chou acids is located in the mitochondria.

11. The method according to claim 5, wherein the recombinant molecule comprises a

¹⁰²
dela gene of *Pseudomonas aeruginosa* that codes for Citrate Synthase.

12. The method according to claim 1, wherein the transcription termination sequence

¹⁰²
dela is the transcription termination sequence of the Nopaline Synthetase gene.

13. The method according to claim 2, wherein the transcription termination sequence

¹⁰²
dela is the transcription termination sequence of the Nopaline Synthetase gene.

14. The method according to claim 3, wherein the transcription termination sequence

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+John is the transcription termination sequence of the Nopaline Synthetase gene.

15. The method according to claim 4, wherein the transcription termination sequence

¹⁰³
Gullum is the transcription termination sequence of the Nopaline Synthetase gene.

16. The method according to claim 5, wherein the transcription termination sequence

¹⁰²
dela is the transcription termination sequence of the Nopaline Synthetase gene.

17. The method according to claim 6, wherein the transcription termination sequence

¹⁰²
dela is the transcription termination sequence of the Nopaline Synthetase gene.

18. The method according to claim 7, wherein the transcription termination sequence

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+Henry is the transcription termination sequence of the Nopaline Synthetase gene.

19. The method according to claim 8, wherein the transcription termination sequence

¹⁰²
dela is the transcription termination sequence of the Nopaline Synthetase gene.

20. The method according to claim 9, wherein the transcription termination sequence

¹⁰³ is the transcription termination sequence of the Nopaline Synthetase gene.

+Chou

- 103 21. The method according to claim 10, wherein the transcription termination sequence is the transcription termination sequence of the Nopaline Synthetase gene.
~~102~~
- 102 22. The method according to claim 1, wherein the promoter is a constitutive promoter.
~~del~~
- 102 23. The method according to claim 2, wherein the promoter is a constitutive promoter.
~~del~~
- 103 24. The method according to claim 3, wherein the promoter is a constitutive promoter.
~~103~~
- 103 25. The method according to claim 4, wherein the promoter is a constitutive promoter.
~~103~~ ~~winner~~
- 102 26. The method according to claim 5, wherein the promoter is a constitutive promoter.
~~del~~
- 102 27. The method according to claim 6, wherein the promoter is a constitutive promoter.
~~del~~
- 103 28. The method according to claim 7, wherein the promoter is a constitutive promoter.
~~103~~ ~~103~~ ~~103~~
- 102 29. The method according to claim 8, wherein the promoter is a constitutive promoter.
~~del~~
- 103 30. The method according to claim 9, wherein the promoter is a constitutive promoter.
~~103~~ ~~103~~
- 103 31. The method according to claim 10, wherein the promoter is a constitutive promoter.
~~103~~ ~~103~~

- 103 32. The method according to claim 1, wherein the promoter is a root-specific promoter.
Delta + Ray
- + John 33. The method according to claim 2, wherein the promoter is a root-specific promoter.
- + Silvana 34. The method according to claim 3, wherein the promoter is a root-specific promoter.
- 103 35. The method according to claim 4, wherein the promoter is a root-specific promoter.
36. The method according to claim 5, wherein the promoter is a root-specific promoter.
37. The method according to claim 6, wherein the promoter is a root-specific promoter.
- 103 38. The method according to claim 7, wherein the promoter is a root-specific promoter.
39. The method according to claim 8, wherein the promoter is a root-specific promoter.
- Chloe 40. The method according to claim 9, wherein the promoter is a root-specific promoter.
- + Chloe 41. The method according to claim 10, wherein the promoter is a root-specific promoter.
- 103 42. The method according to claim 1, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

43. The method according to claim 2, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

John 44. The method according to claim 3, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

+ Saluman 45. The method according to claim 4, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

46. The method according to claim 5, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

47. The method according to claim 6, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

103 John 48. The method according to claim 7, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

49. The method according to claim 8, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

Khoa 50. The method according to claim 9, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

Khoa 51. The method according to claim 10, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

103 Crismon 52. The method according to claim 1, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

53. The method according to claim 2, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

Cu 103 + John

54. The method according to claim 3, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

+ S. John 55. The method according to claim 4, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

56. The method according to claim 5, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

57. The method according to claim 6, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

103 John 58. The method according to claim 7, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

59. The method according to claim 8, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

Klara 60. The method according to claim 9, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

Clara 61. The method according to claim 10, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

102 62. The method according to claim 1, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

102 Clara 63. The method according to claim 2, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

103 64. The method according to claim 3, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

+ John

+ Silverman

163 65. The method according to claim 4, wherein the promoter is the 35S promoter of
the cauliflower mosaic virus.

102 66. The method according to claim 5, wherein the promoter is the 35S promoter of
Dela the cauliflower mosaic virus.

102 67. The method according to claim 6, wherein the promoter is the 35S promoter of
Dela the cauliflower mosaic virus.

102 68. The method according to claim 7, wherein the promoter is the 35S promoter of
the cauliflower mosaic virus.

102 69. The method according to claim 8, wherein the promoter is the 35S promoter of
the cauliflower mosaic virus.

102 70. The method according to claim 9, wherein the promoter is the 35S promoter of
the cauliflower mosaic virus.

102 71. The method according to claim 10, wherein the promoter is the 35S promoter of
the cauliflower mosaic virus.

103 72. The method according to claim 9, wherein the recombinant molecule comprises a
signal peptide sequence to direct a heterologous enzyme that synthesizes organic acids to the
chloroplast or the mitochondria of the transgenic cells.

103 73. The method according to claim 10, wherein the recombinant molecule comprises
a signal peptide sequence to direct a heterologous enzyme that synthesizes organic acids to the
chloroplast or the mitochondria of the transgenic cells.

102! 74. A recombinant heterologous DNA molecule comprising one or more genes that
code for enzymes that synthesize organic acids, functionally linked to a promoter sequence
functional in plants, and to a transcription termination/polyadenylation sequence functional in
plants.

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102 75. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids, is from one or more microbial genes.

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103 76. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids is from a gene of plant origin.

102
+ Sherman 77. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids is from a gene of animal origin.

102
102 78. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids, is from one or more bacterial genes.

102
Delta 79. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is a gene that codes for the enzyme citrate synthase.

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Delta 80. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is a gene of *Pseudomonas aeruginosa* that codes for the enzyme citrate synthase.

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Ubu 81. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is a gene that codes for the enzyme Malate deshydrogenase. *SD*

102
Delta 82. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is an enzyme that is located in the cytoplasm.

103
+ Aron 83. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is an enzyme that is located in the chloroplast.

(103) 84. The recombinant DNA molecule according to claim 74, wherein the promoter is a constitutive promoter.

+Croy 85. The recombinant DNA molecule according to claim 74, wherein the promoter is a root-specific promoter.

+ 103 Nucleo 86. The recombinant DNA molecule according to claim 74, wherein the promoter is a promoter whose expression is inducible by stress caused by low phosphate availability.

+ 103 RC promoter 87. The recombinant DNA molecule according to claim 74, wherein the promoter is a promoter whose expression is inducible by stress caused by low iron availability.

Dna 88. The recombinant DNA molecule according to claim 74, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

+ 103 plants 89. The recombinant DNA molecule according to claim 74 comprising a sequence that includes a transit peptide sequence for chloroplast or mitochondrial protein targeting in plants.

+ 102 Dna 90. The recombinant DNA molecule according to claim 74, comprising a transcription termination/polyadenylation sequence that is the transcription termination/polyadenylation sequence of the Nopaline Synthetase gene.

102 Dna 91. The recombinant DNA molecule according to claim 74, as defined in figure 1.

102 Dna 93. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 74.

102 Dna 94. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 75.

102 95. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 76.

103 96. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 77.

+5 hours 102 97. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 78.

102 98. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 79.

102 99. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 80.

103 100. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 81.

102 101. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 82.

103 102. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 83.

Chao

- ~~102~~ ¹⁰³ 114. Transgenic plants according to claim 93, tolerant to toxic concentrations of Aluminum.
- ~~102~~ ¹⁰⁴ ~~112~~ 112. Transgenic plants according to claim 93, having increased capacity to solubilize or accumulate phosphate.
- ~~103~~ ¹⁰⁵ ~~113~~ 113. Transgenic plants according to claim 93, having increased capacity to solubilize or accumulate iron.
- ~~102~~ ¹⁰⁶ ~~114~~ 114. Transgenic plants according to claim 93, requiring less fertilizer for their growth.
- ~~102~~ ¹⁰⁷ ~~115~~ 115. Transgenic plants according to claim 93, that develop better or have higher productivity in acid soils.
- ~~102~~ ¹⁰⁸ ~~116~~ 116. The transgenic plants according to claim 93, wherein the plant is a monocotyledonous plant.
- ~~102~~ ¹⁰⁹ ~~117~~ 117. Transgenic plants according to claim 93, wherein the plant is a dicotyledonous plant.
- ~~103~~ ¹¹⁰ ~~118~~ 118. Transgenic plants according to claim 115, wherein the plant belongs to anyone of the families: Poaceae or Liliaceae.
- ~~103~~ ¹¹¹ ~~119~~ 119. Transgenic plants according to claim 117, wherein the plant belongs to anyone of the families: Leguminosae, Solanaceae, Caricaceae or Cucurbitaceae.
- ~~103~~ ¹¹² ~~120~~ 120. Transgenic plants according to claim 116, wherein the plant belongs to any of the species: Triticum spp, Oryza sativa, Zea mays, Sorghum bicolor, Avena sativa or Saccharum officinarum.
- ~~103~~ ¹¹³ ~~121~~ 121. Transgenic plants according to claim 117, wherein the plant belongs to any of the species: Solanum tuberosum, Lycopersicum esculentum or Glycine max.

*112 and
compared
to wheat?*

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102 114 122. Transgenic plants according to claim 117, wherein the plant is of the Nicotiana genus.

102 115 123. Transgenic plants according to claim 122, wherein the plant is of the Nicotiana tabacum species.

102 116 124. Transgenic plants according to claim 117, wherein the plant is of the Carica genus.

102 117 125. Transgenic plants according to claim 124, wherein the plant is of the Carica papaya species.

103 118 126. Use of the transgenic plants according to claim 81 in acid soils.

103 119 127. Use of the transgenic plants according to claim 81 in soils containing phosphates in forms not available for the plant nutrition.

103 120 128. Use of the transgenic plants according to claim 81, for practice or cultivation systems that use less fertilizer.

103 121 129. The transgenic seeds or any vegetative reproductive structure attainable from a transgenic plant as defined in the claim 81.

102 122 130. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 74.

102 123 131. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 75.

103 124 132. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 76.

103 125 133. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 77.

¹²⁶
134 A transformed cell or protoplast transformed with the recombinant DNA molecule

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as defined in claim 78.

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135 A transformed cell or protoplast transformed with the recombinant DNA molecule

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as defined in claim 79.

¹²⁶
136 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 80.

¹²⁶
137 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 81.

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138 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 82.

¹³⁾
139 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 83.

¹³²
140 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 81.

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141 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 85.

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142 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 86.

¹³⁵
143 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 87.

¹³⁶
144 A transformed cell or protoplast transformed with the recombinant DNA molecule

as defined in claim 88.

- b3* 13> 145. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 89.
- tell ref 138* 10) 146. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 90.